Attachment 5

The drawings submitted with this application were declared informal by the applicant. Accordingly they have not been reviewed by a draftsperson at this time. When formal drawings are submitted, the draftsperson will perform a review.

Direct any inquires concerning drawing review to the Drawing Review Branch (703) 305-8404.

FIGURE 1

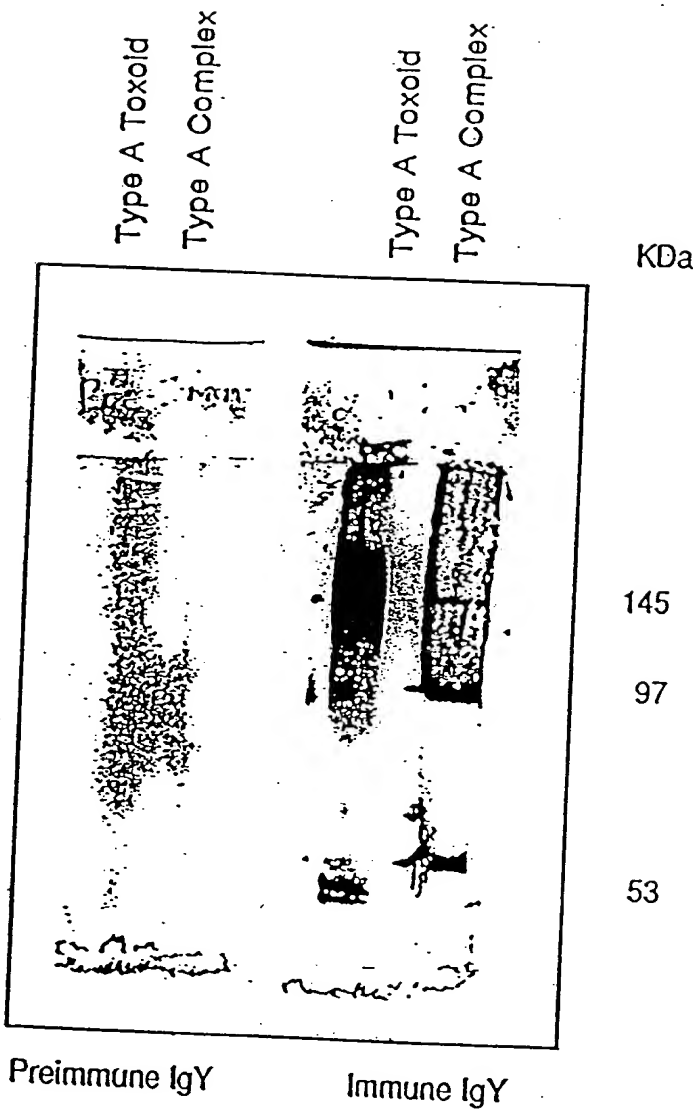

Type A Toxoid
Type A Complex
Type A Toxoid
Type A Complex
KDa
145
97
53
Preimmune IgY
Immune IgY

FIGURE 2

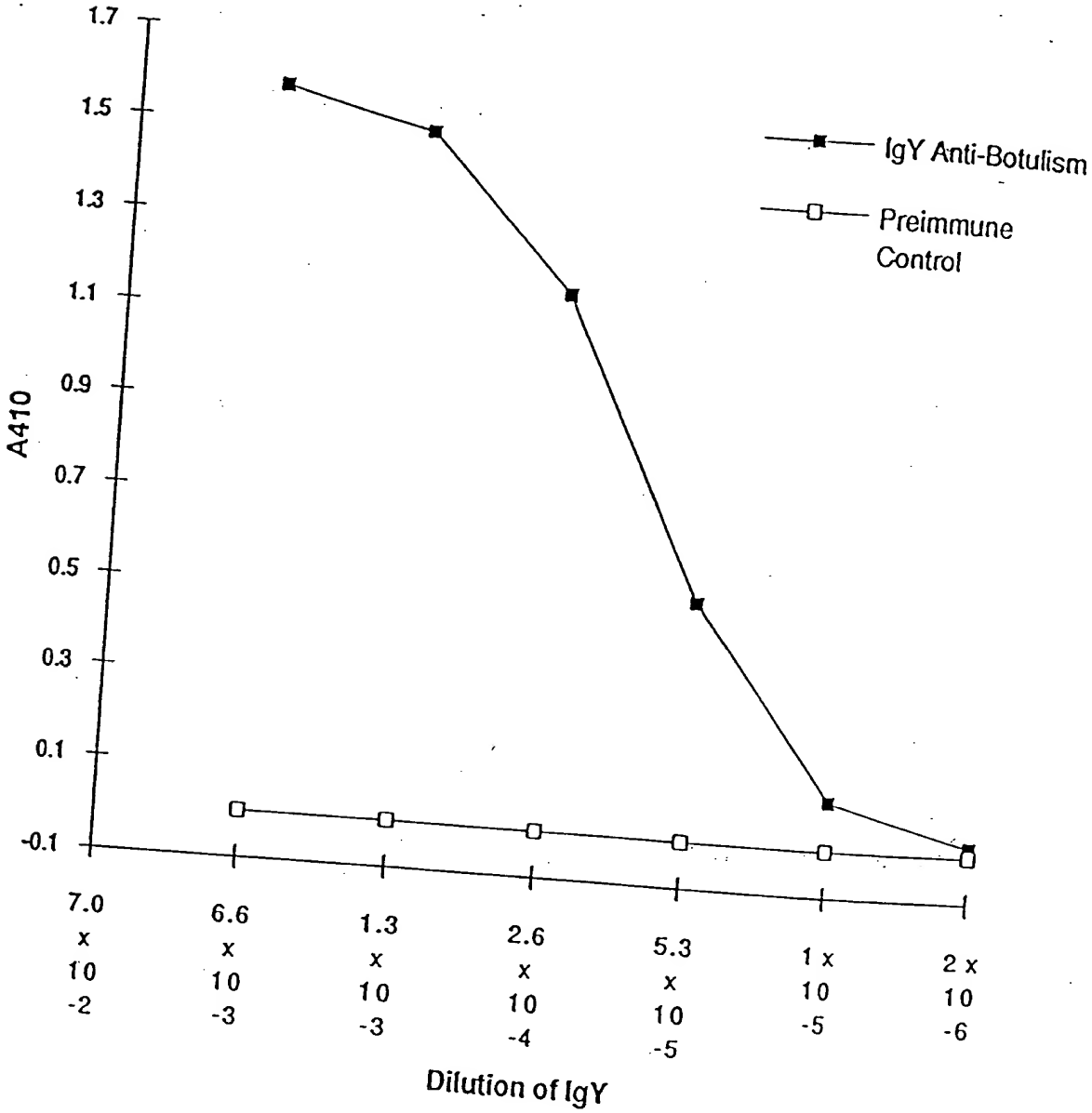

1.7
1.5
1.3
1.1
0.9
0.7
0.5
0.3
0.1
-0.1
A410
IgY Anti-Botulism
Preimmune Control
7.0 x 10 -2
6.6 x 10 -3
1.3 x 10 -3
2.6 x 10 -4
5.3 x 10 -5
1 x 10 -5
2 x 10 -6
Dilution of IgY

FIGURE 3

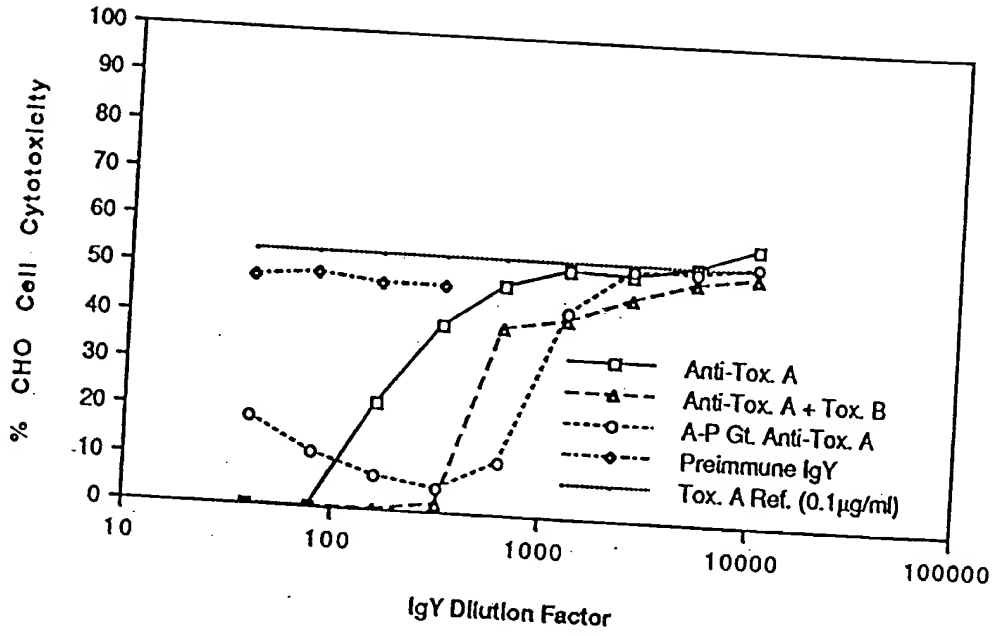

% CHO Cell Cytotoxicity
100
90
80
70
60
50
40
30
20
10
0
10
100
1000
10000
100000
IgY Dilution Factor
Anti-Tox. A
Anti-Tox. A + Tox. B
A-P Gt. Anti-Tox. A
Preimmune IgY
Tox. A Ref. (0.1µg/ml)

FIGURE 4

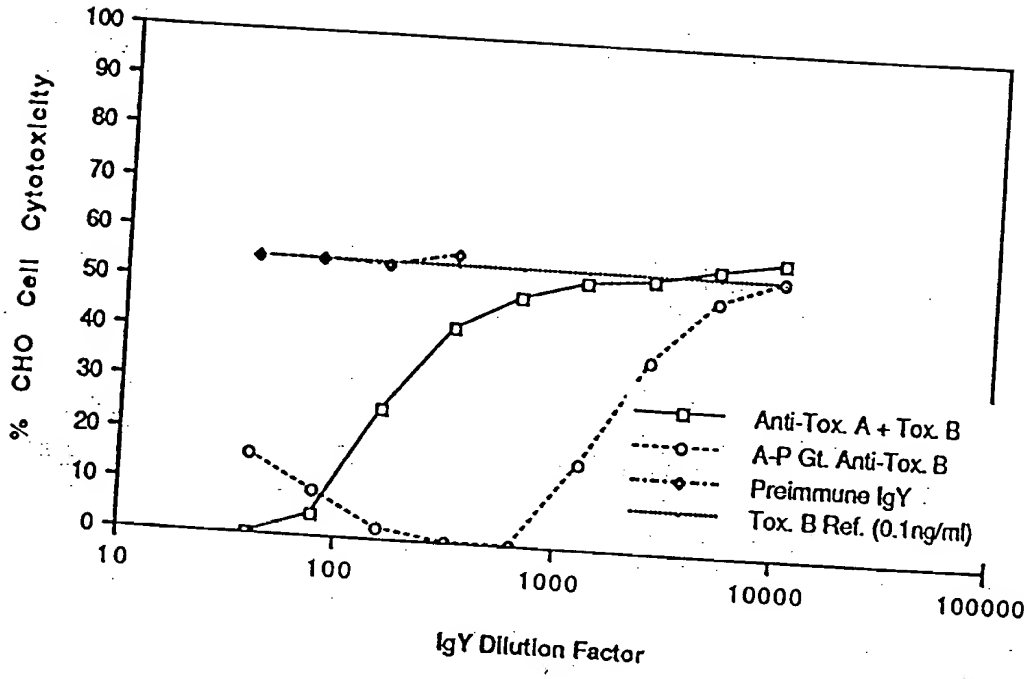

% CHO Cell Cytotoxicity
100
90
80
70
60
50
40
30
20
10
0
10
100
1000
10000
100000
IgY Dilution Factor
Anti-Tox. A + Tox. B
A-P Gt. Anti-Tox. B
Preimmune IgY
Tox. B Ref. (0.1ng/ml)

FIGURE 5

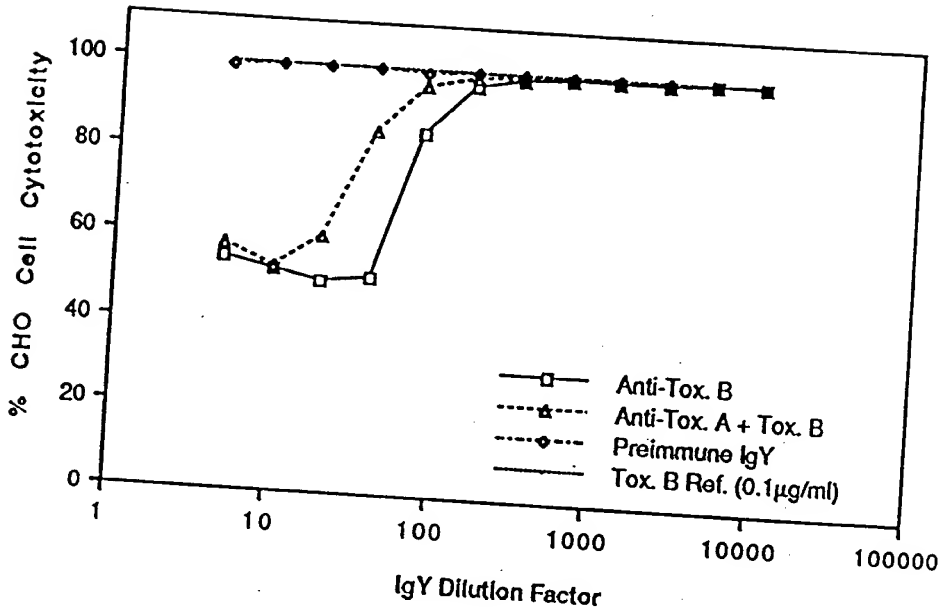
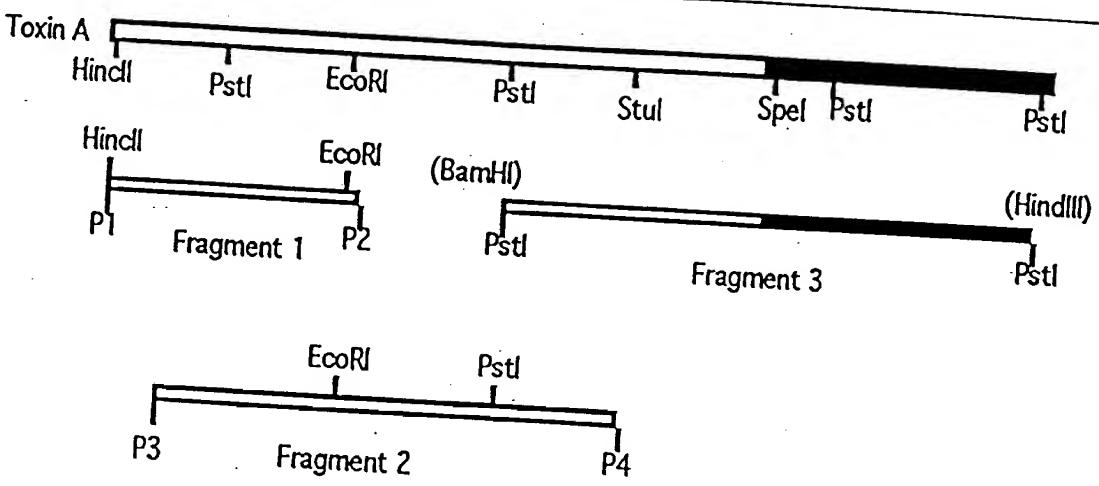

% CHO Cell Cytotoxicity
100
80
60
40
20
0
1
10
100
1000
10000
100000
IgY Dilution Factor
Anti-Tox. B
Anti-Tox. A + Tox. B
Preimmune IgY
Tox. B Ref. (0.1µg/ml)

FIGURE 6



P1-P4 are PCR primers 1-4. P1=5'GGAAATTTAGCTGCAGCATCTGAC3', P2=5'TCTAGCAAATTCGCTTGTGTTGAA3',P3=5'CTCGCATATAGCATTAGACC3', P4=5'CTATCTAGGCCTAAAGTAT3'. Indicated restriction sites in fragments 1 and 2 are internal sites used to clone into pGEX2T vector (fragment 1; construct called pGA30-660) or pMALc vector (fragment 2; construct called pMA660-1100). Bracketed restriction sites at ends of fragment 3 are pUC9 polylinker sites utilized to clone fragment 3 into pET23 vector (construct called pPA1100-2680). Numbers in these constructs refer to toxin A amino acid interval that is expressed. The shaded portion of the toxin A gene corresponds to the repeating ligand binding domain.

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FIGURE 7

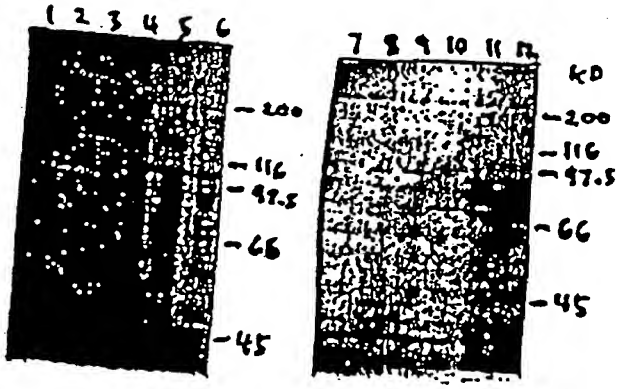
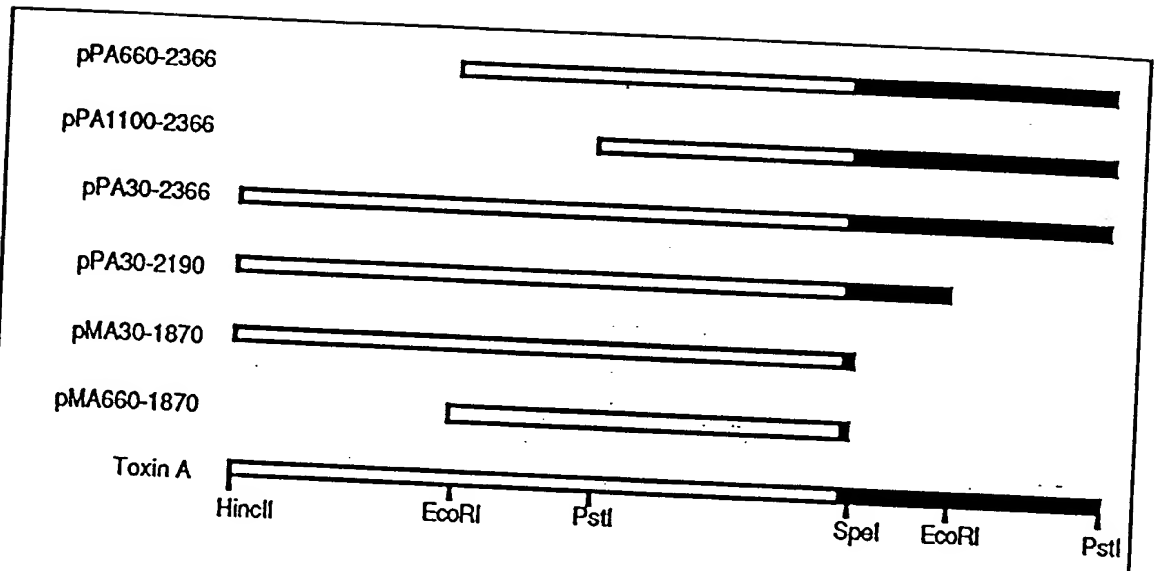
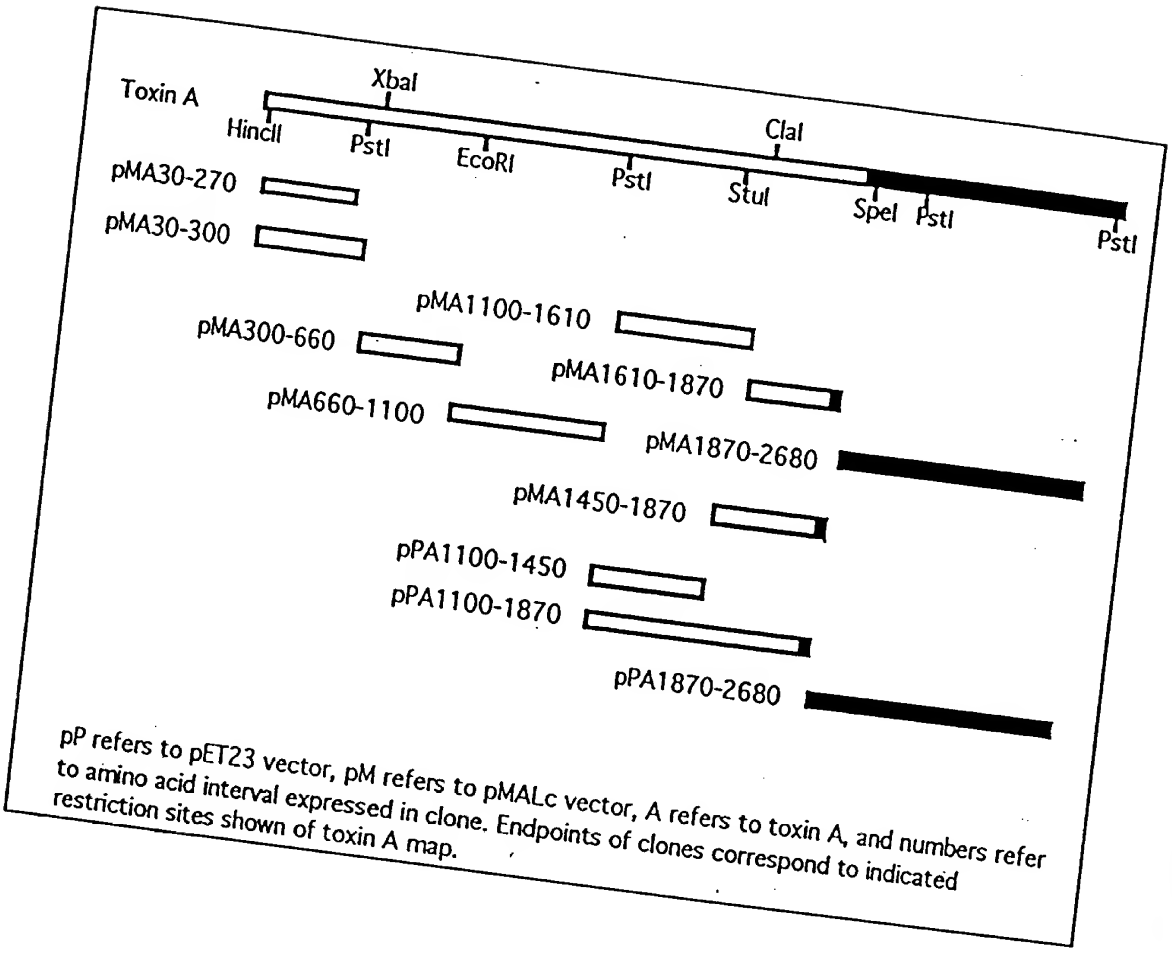


FIGURE 8



pP refers to pET23 vector, pM refers to pMALc vector, A refers to toxin A, and numbers refer to amino acid interval expressed in clone. Endpoints of clones correspond to indicated restriction sites shown of toxin A map.

FIGURE 9



pP refers to pET23 vector, pM refers to pMALc vector, A refers to toxin A, and numbers refer to amino acid interval expressed in clone. Endpoints of clones correspond to indicated restriction sites shown of toxin A map.

70459

FIGURE 10

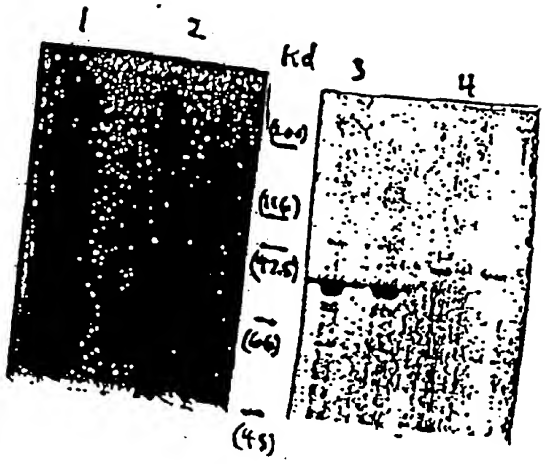


FIGURE 11

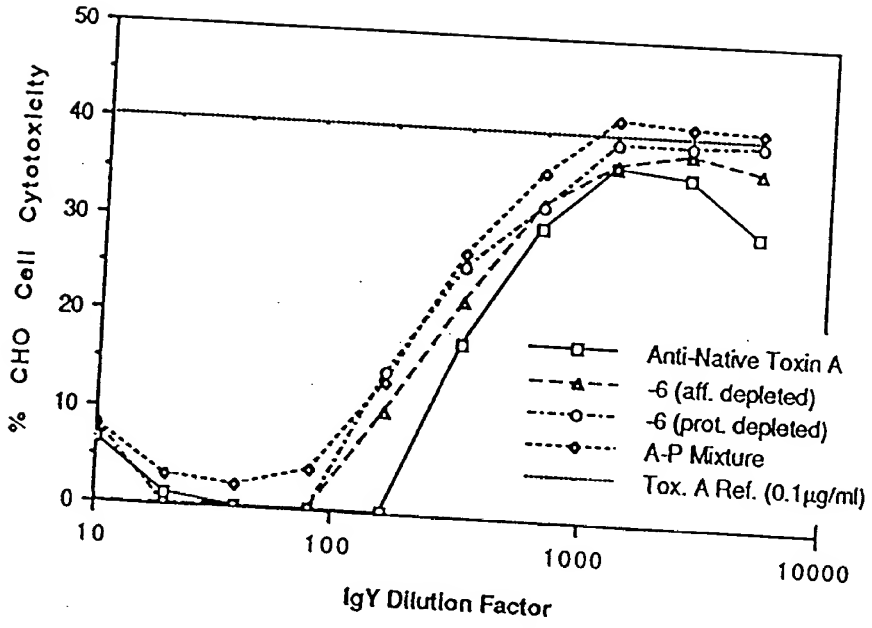

50
40
30
20
10
0
% CHO Cell Cytotoxicity
10
100
1000
10000
IgY Dilution Factor
Anti-Native Toxin A
-6 (aff. depleted)
-6 (prot. depleted)
A-P Mixture
Tox. A Ref. (0.1µg/ml)

FIGURE 12

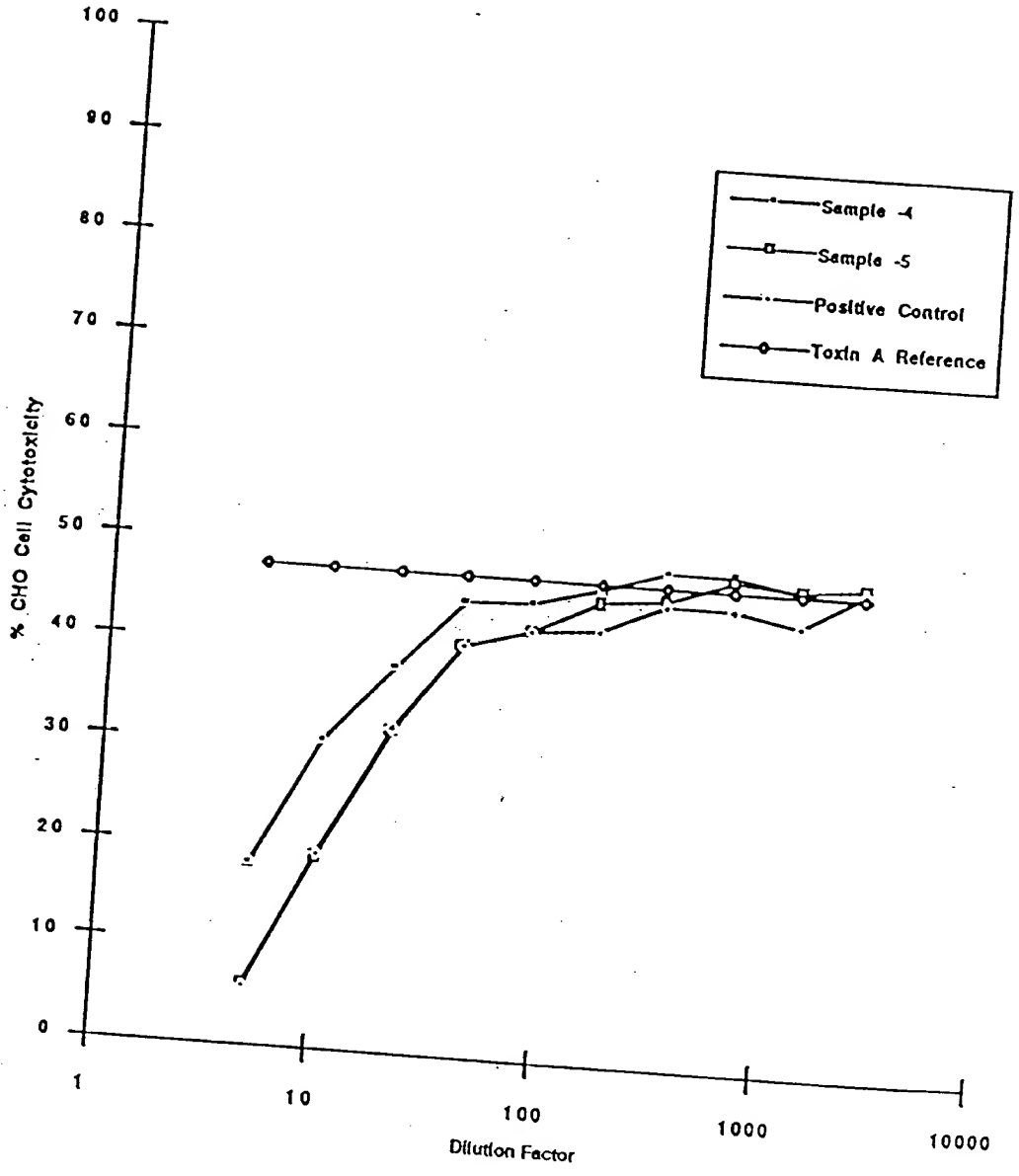

100
90
80
70
60
50
40
30
20
10
0
% CHO Cell Cytotoxicity
1
10
100
1000
10000
Dilution Factor
Sample -4
Sample -5
Positive Control
Toxin A Reference

FIGURE 13

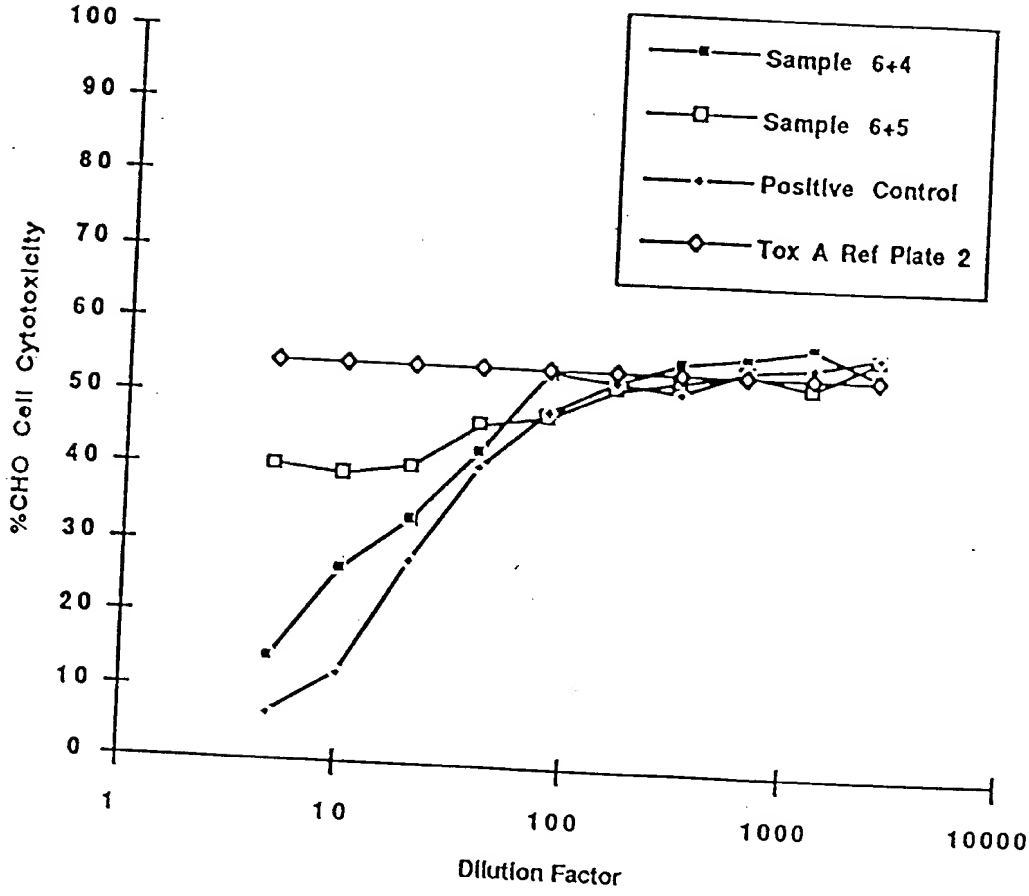

100
90
80
70
60
50
40
30
20
10
0
%CHO Cell Cytotoxicity
1
10
100
1000
10000
Dilution Factor
Sample 6+4
Sample 6+5
Positive Control
Tox A Ref Plate 2

FIGURE 14

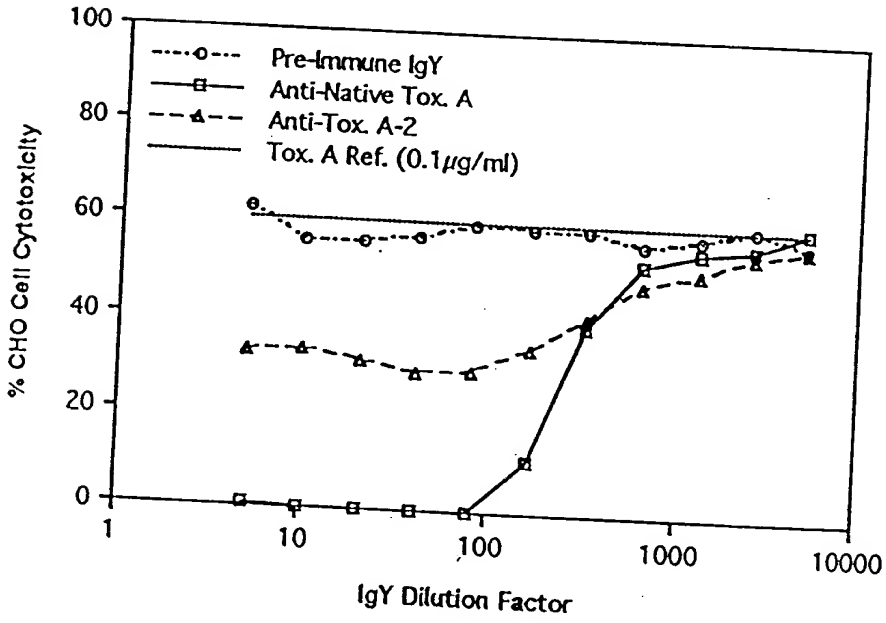

100
80
60
40
20
0
% CHO Cell Cytotoxicity
Pre-Immune IgY
Anti-Native Tox. A
Anti-Tox. A-2
Tox. A Ref. (0.1μg/ml)
1
10
100
1000
10000
IgY Dilution Factor

FIGURE 15

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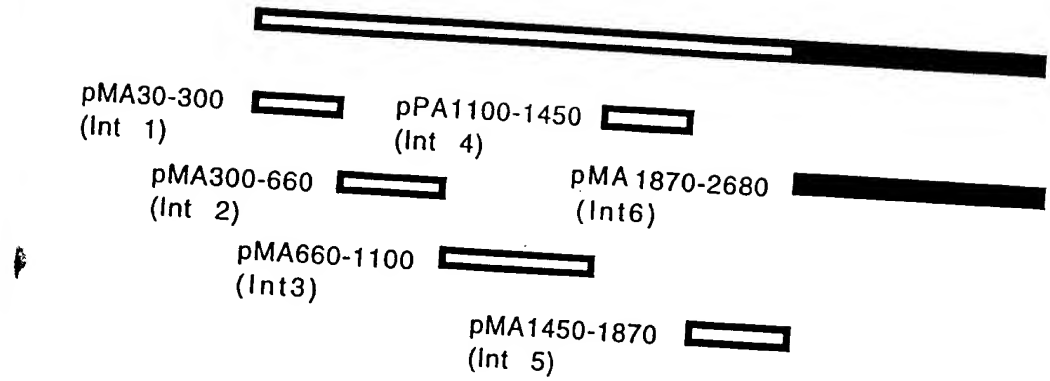
A



B

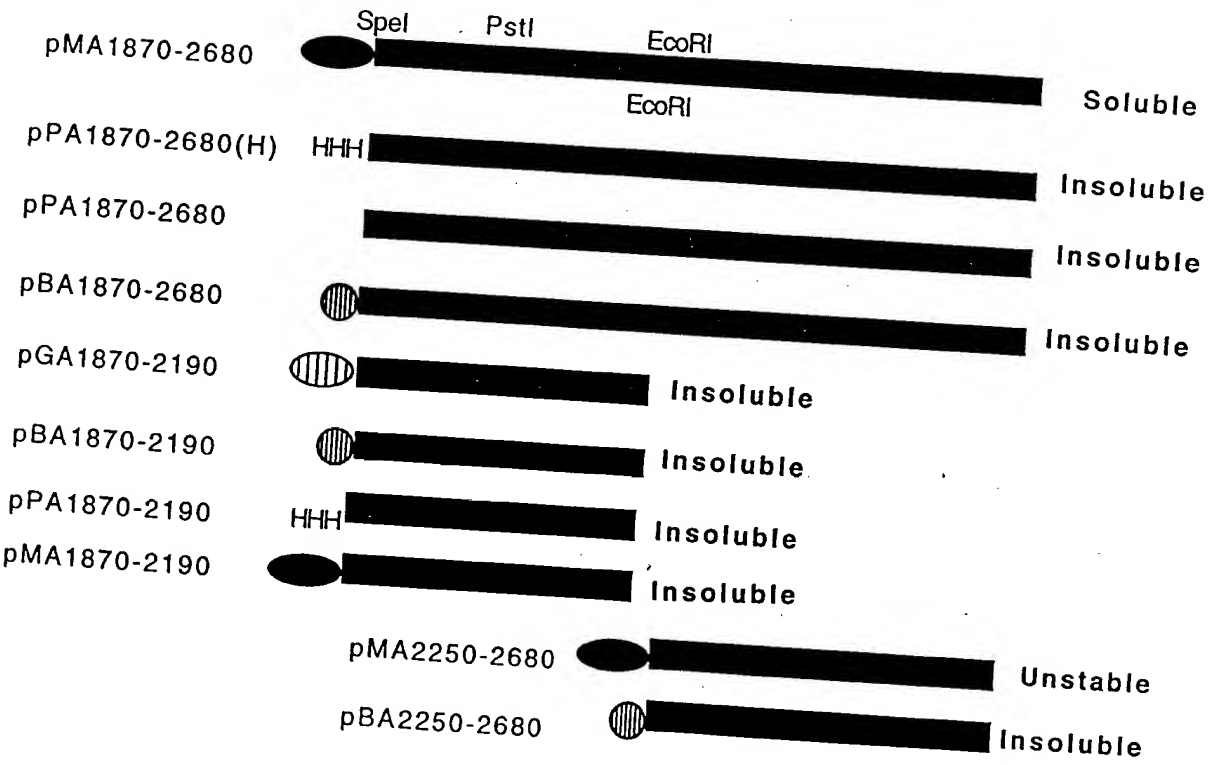


FIGURE 16

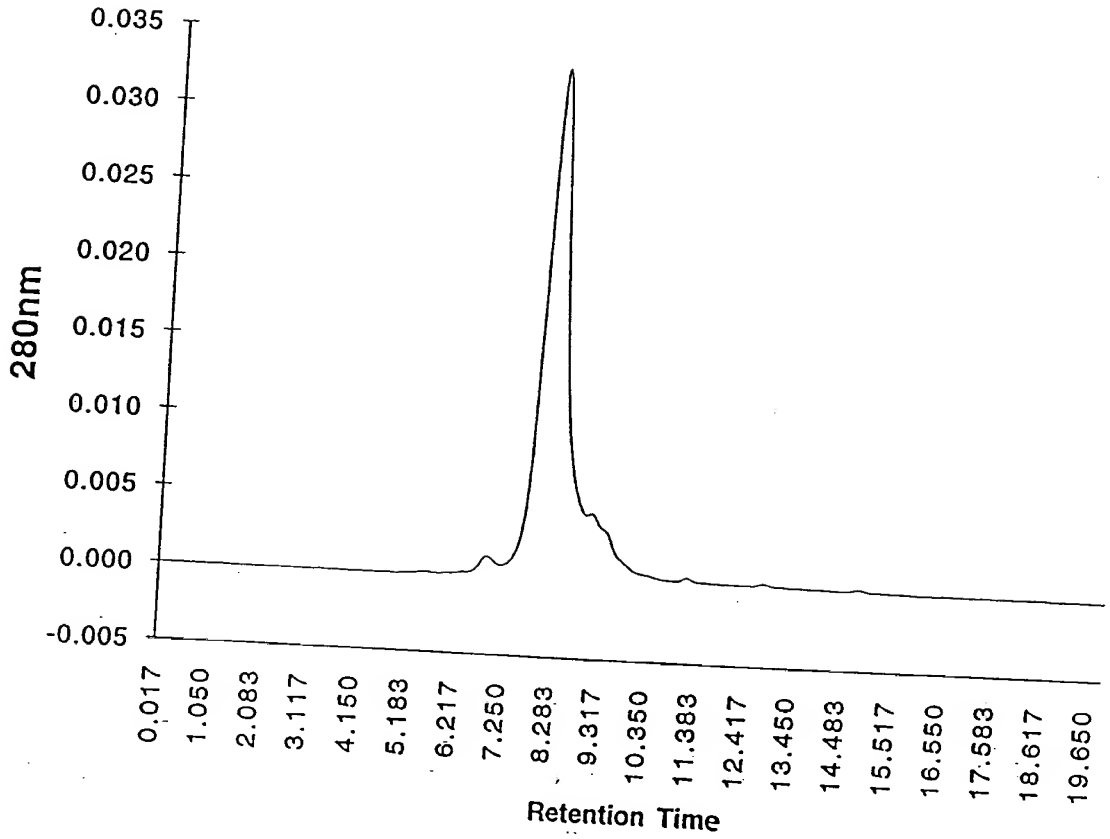

0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
-0.005
280nm
0.017
1.050
2.083
3.117
4.150
5.183
6.217
7.250
8.283
9.317
10.350
11.383
12.417
13.450
14.483
15.517
16.550
17.583
18.617
19.650
Retention Time

FIGURE 17

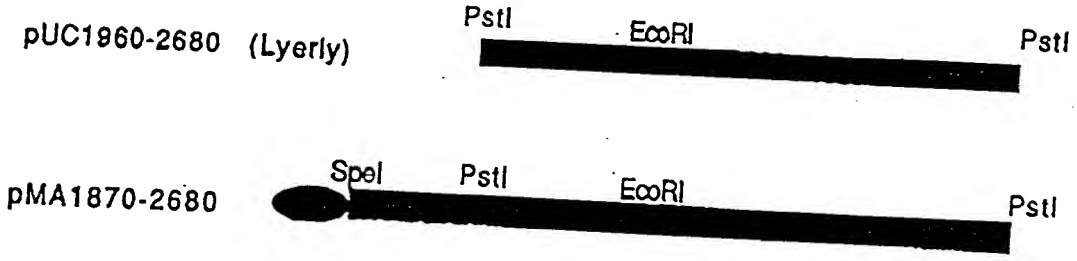

pUC1960-2680 (Lyerly)
PstI
EcoRI
PstI
pMA1870-2680
SpeI
PstI
EcoRI
PstI

FIGURE 18

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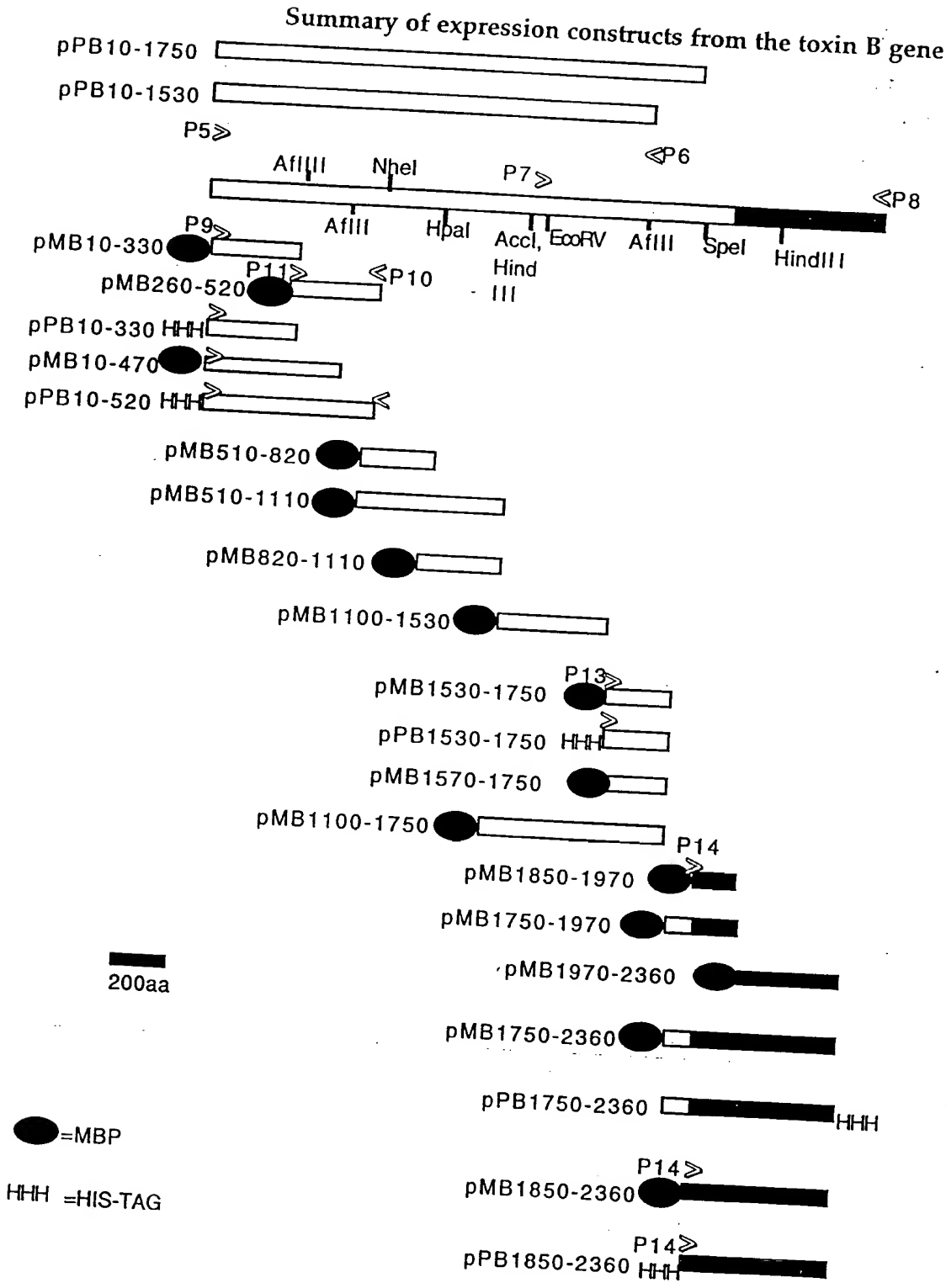

FIGURE 19

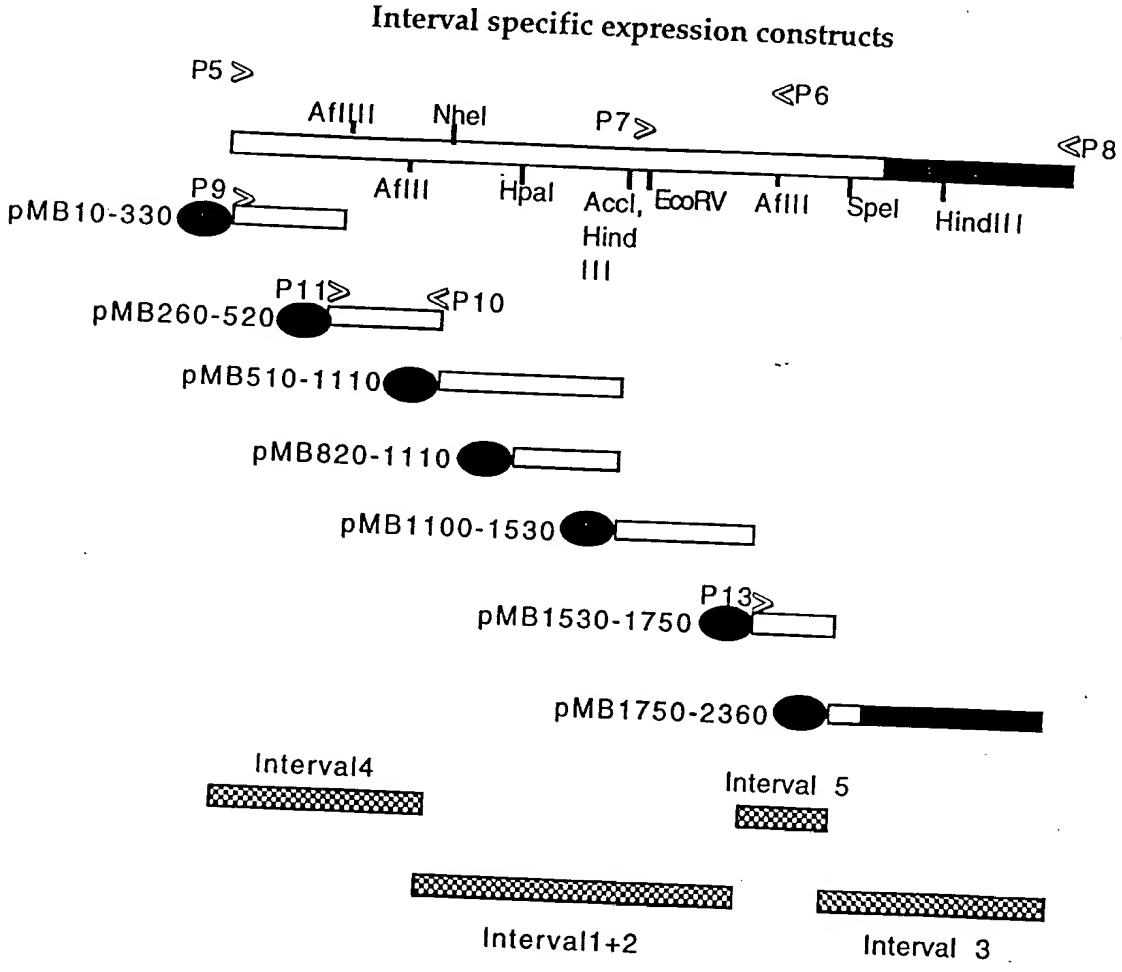
Interval specific expression constructs
P5
AflIII
NheI
P7
P6
P8
P9
AflII
HpaI
AccI, HindIII
EcoRV
AflII
SpeI
HindIII
pMB10-330
P11
P10
pMB260-520
pMB510-1110
pMB820-1110
pMB1100-1530
P13
pMB1530-1750
pMB1750-2360
Interval4
Interval 5
Interval1+2
Interval 3

FIGURE 20

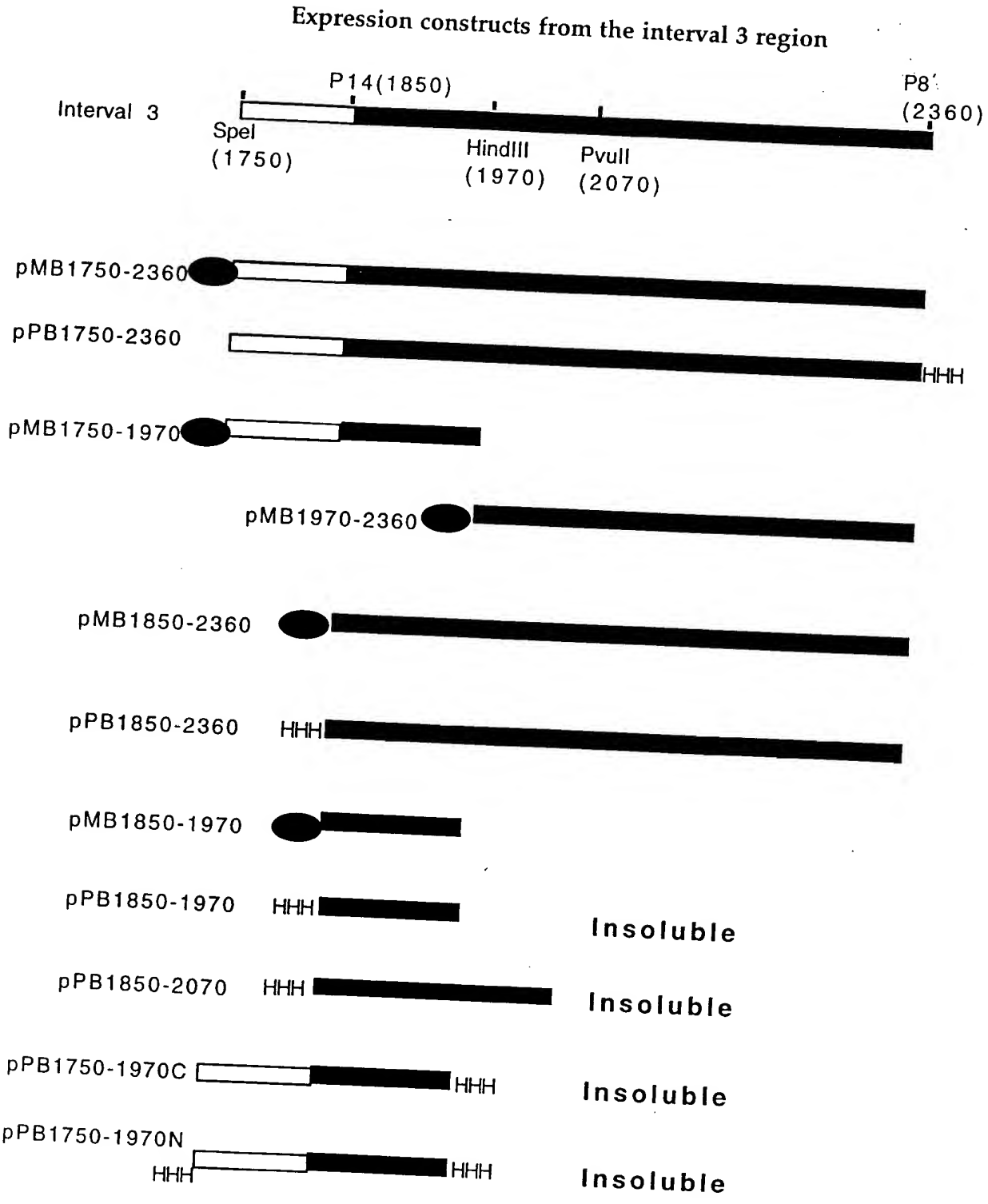
Expression constructs from the interval 3 region
Interval 3
P14(1850)
P8'
(2360)
SpeI
(1750)
HindIII
(1970)
PvuII
(2070)
pMB1750-2360
pPB1750-2360
HHH
pMB1750-1970
pMB1970-2360
pMB1850-2360
pPB1850-2360
HHH
pMB1850-1970
pPB1850-1970
HHH
Insoluble
pPB1850-2070
HHH
Insoluble
pPB1750-1970C
HHH
Insoluble
pPB1750-1970N
HHH
HHH
Insoluble

FIGURE 21

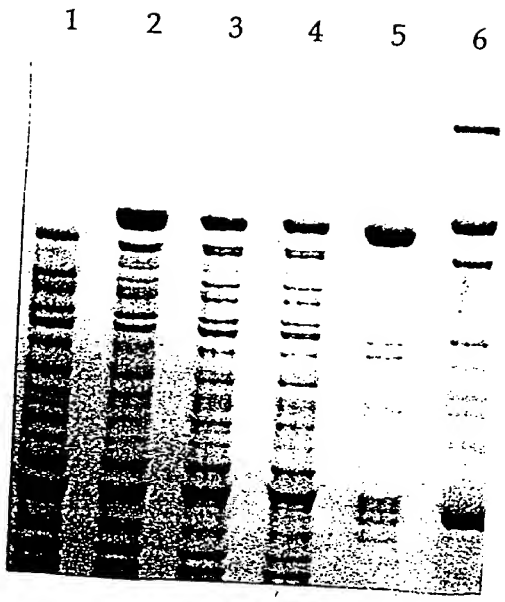

FIGURE 22

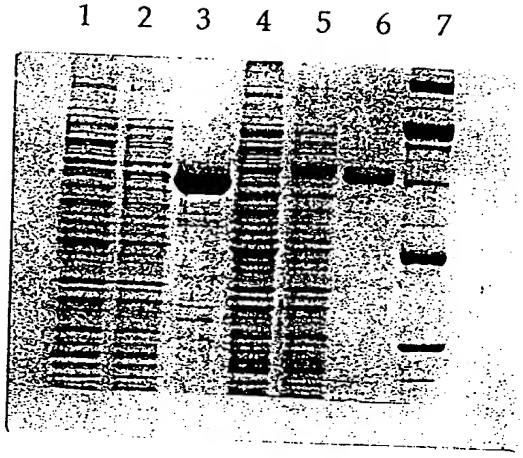

FIGURE 23

Binding of neutralizing CTB antibodies by recombinant toxin B protein

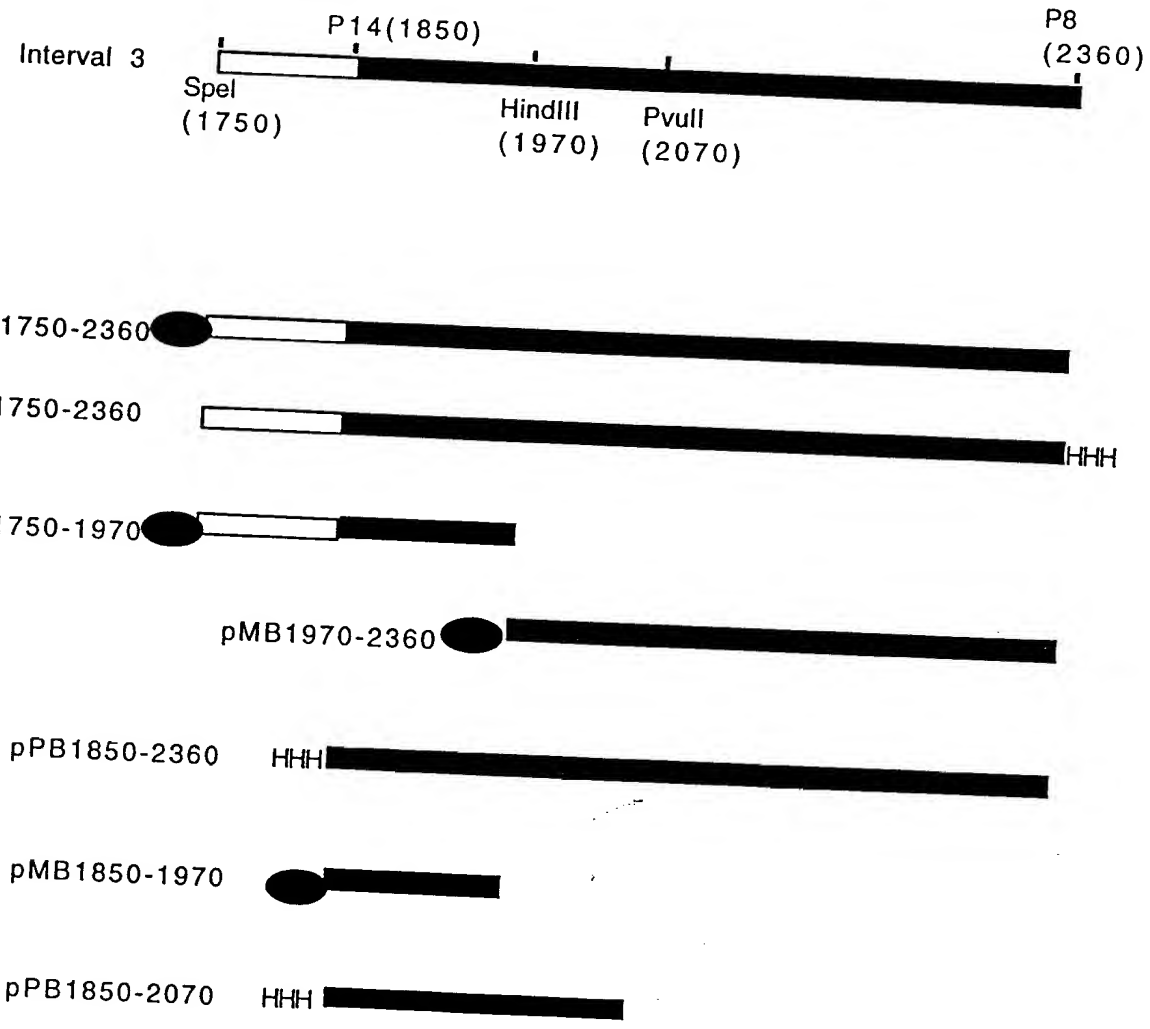

FIGURE 24

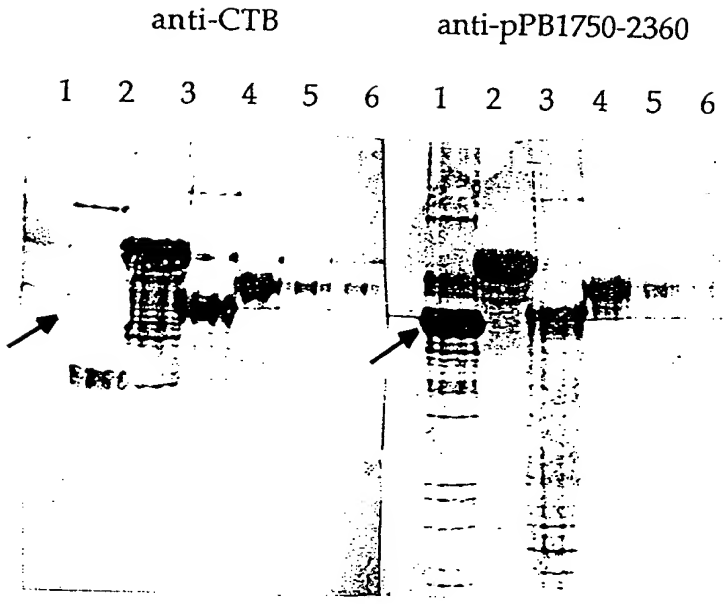

FIGURE 25

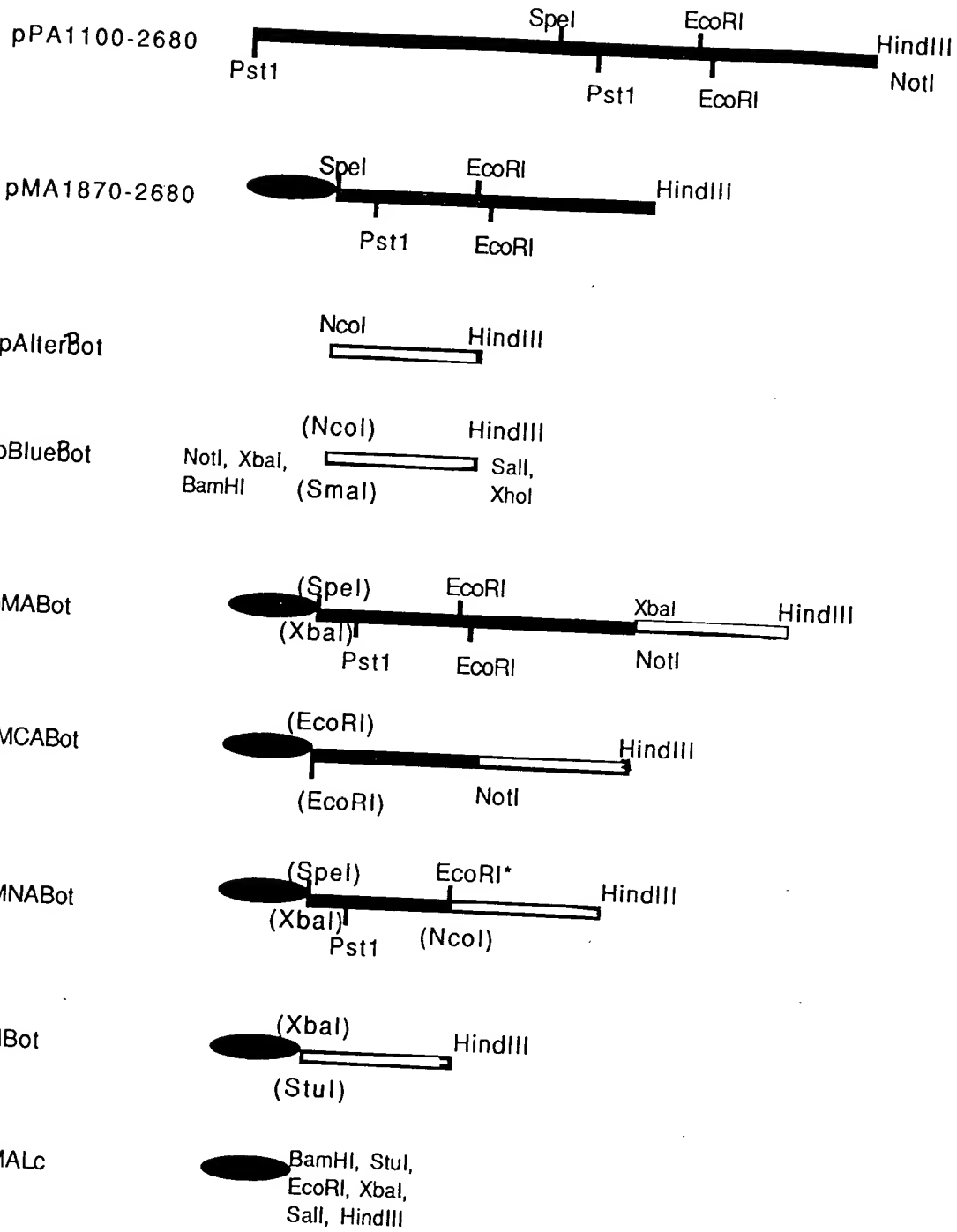
pPA1100-2680
SpeI
EcoRI
HindIII
Pst1
Pst1
EcoRI
NotI
pMA1870-2680
SpeI
EcoRI
HindIII
Pst1
EcoRI
pAlterBot
NcoI
HindIII
pBlueBot
(NcoI)
HindIII
NotI, XbaI, BamHI
(SmaI)
SalI, XhoI
pMABot
(SpeI)
EcoRI
XbaI
HindIII
(XbaI)
Pst1
EcoRI
NotI
pMCABot
(EcoRI)
HindIII
(EcoRI)
NotI
pMNABot
(SpeI)
EcoRI*
HindIII
(XbaI)
Pst1
(NcoI)
pMBot
(XbaI)
HindIII
(StuI)
pMALc
BamHI, StuI, EcoRI, XbaI, SalI, HindIII

FIGURE 26

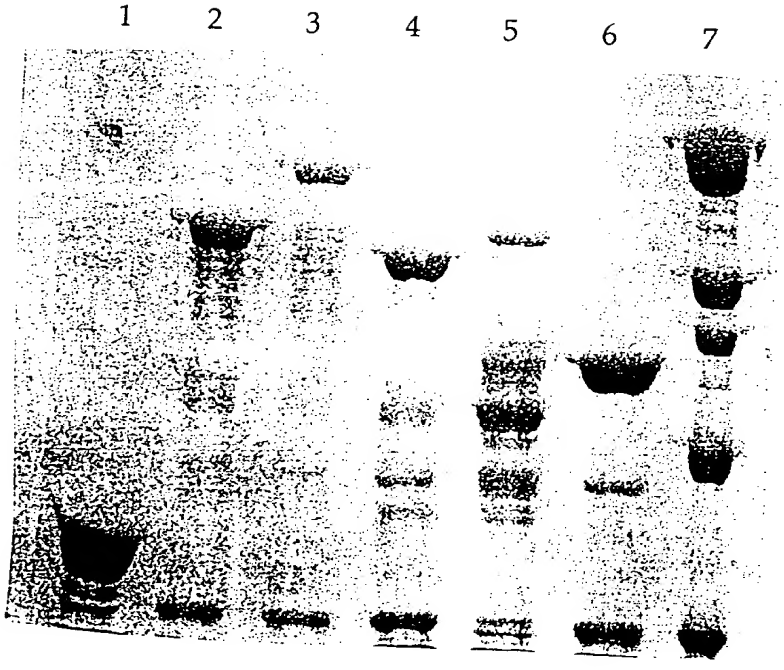

FIGURE 27

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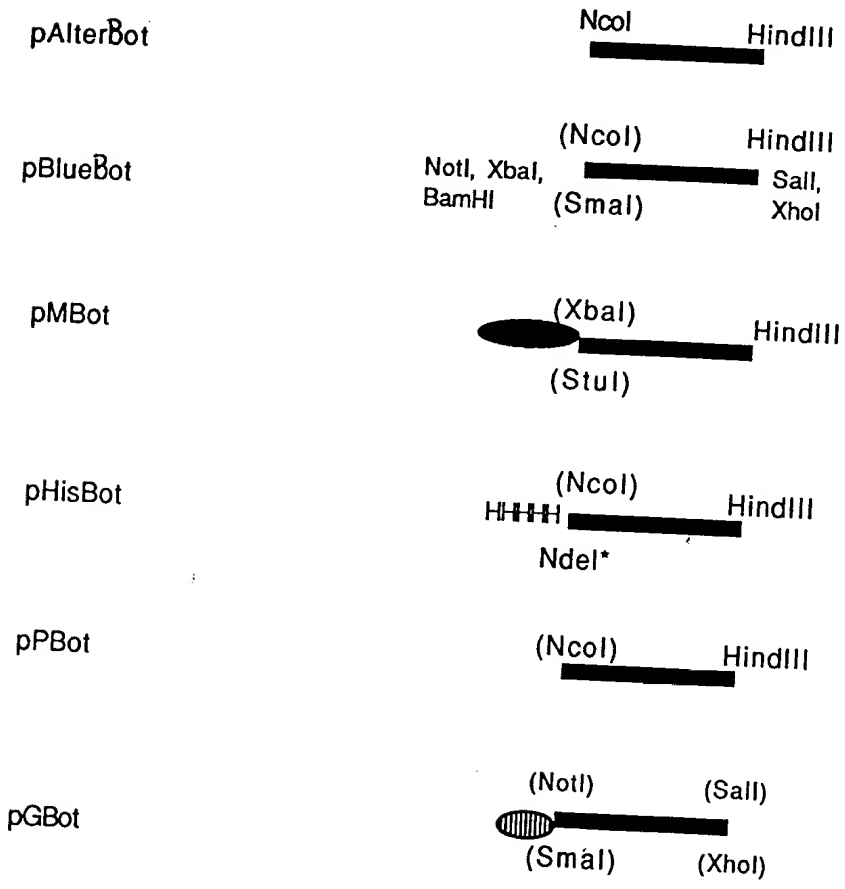


FIGURE 28

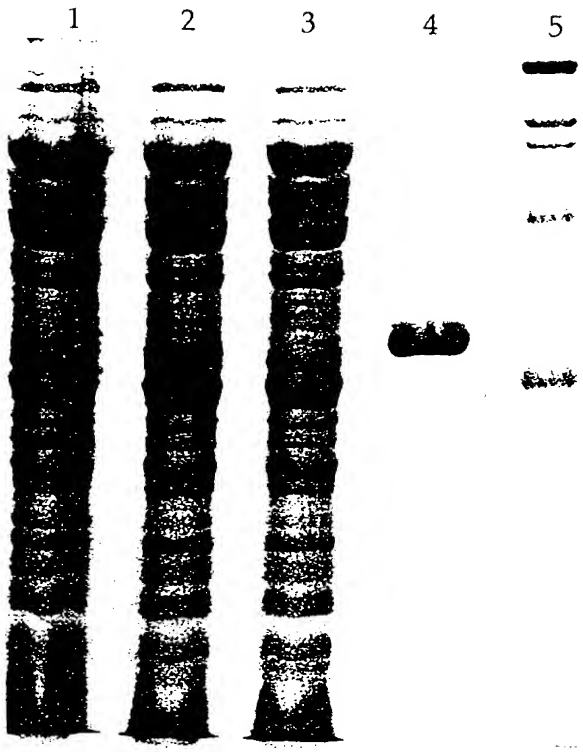


FIGURE 29

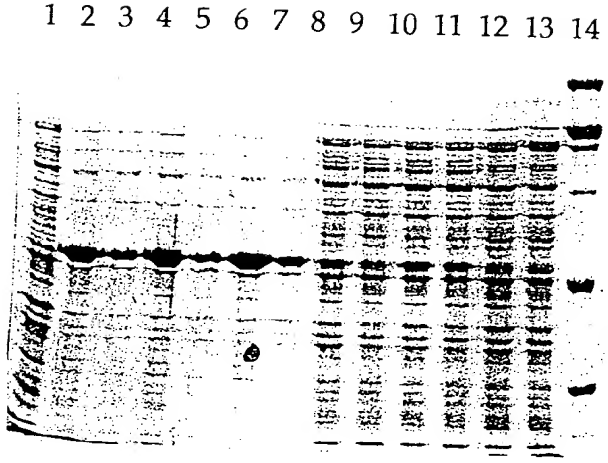
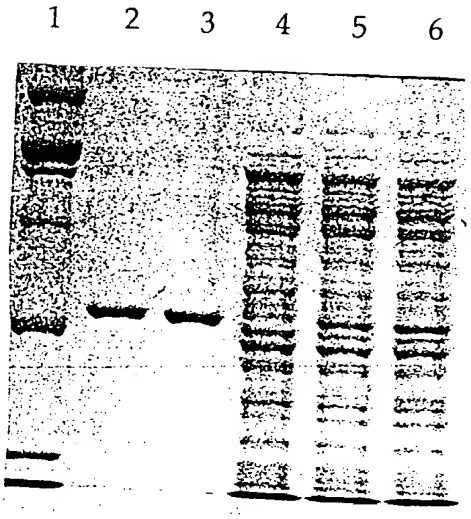


FIGURE 30



08 704159

FIGURE 31

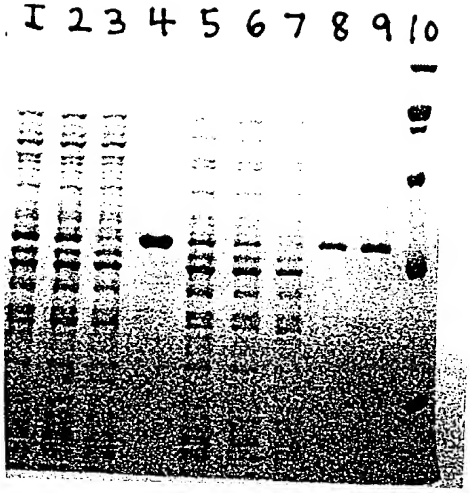


FIGURE 32

08 704159

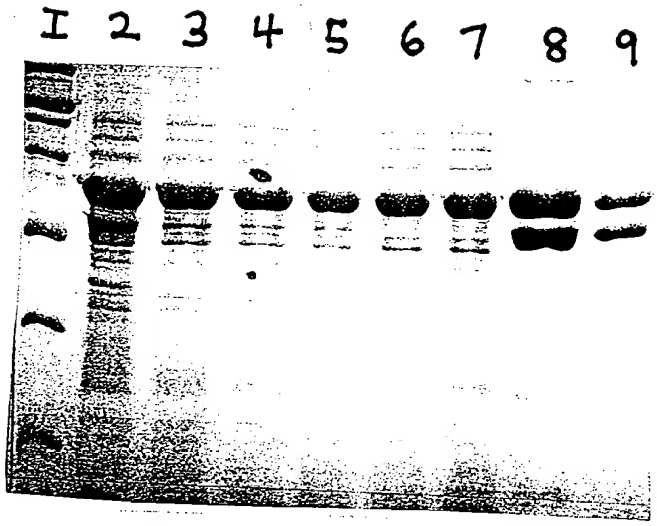


FIGURE 33

08 704159

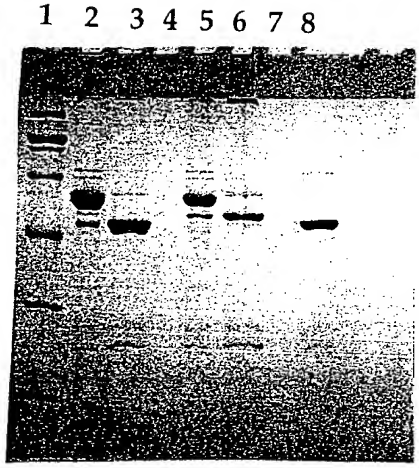


FIGURE 34

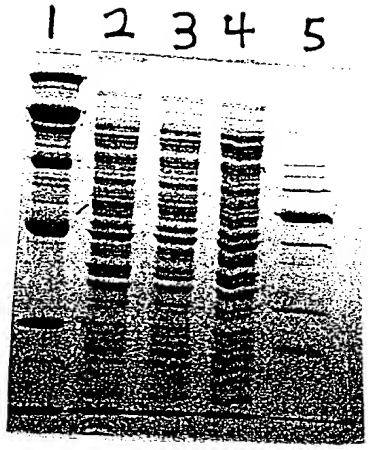
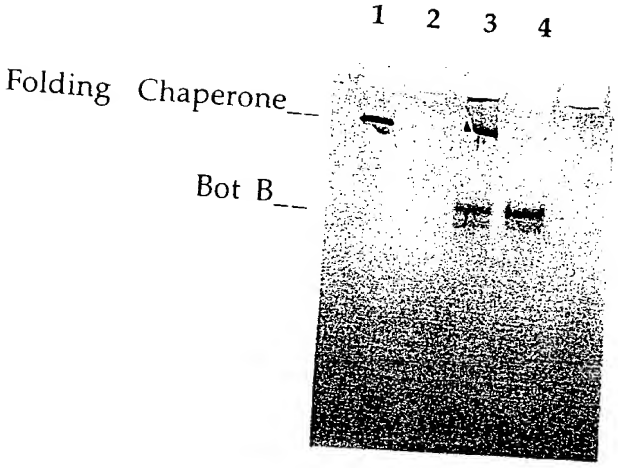
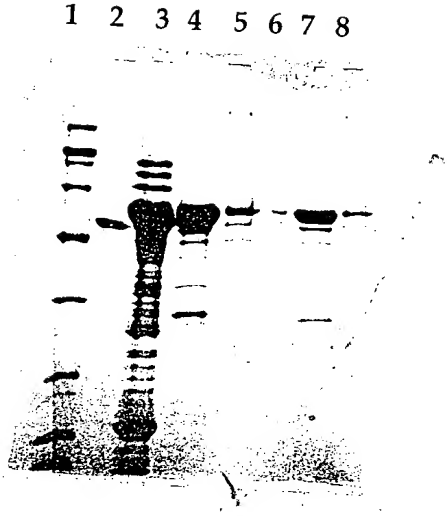


FIGURE 35



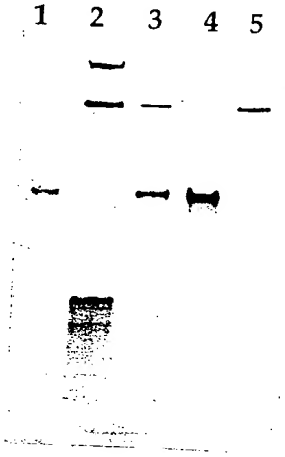
08 704159

FIGURE 36



08 704159

FIGURE 37



08 704159

FIGURE 38

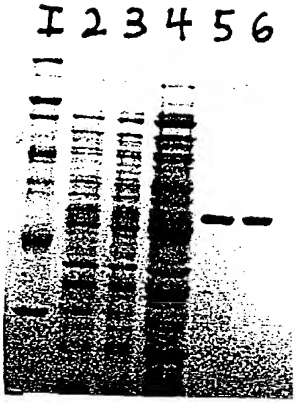


FIGURE 39

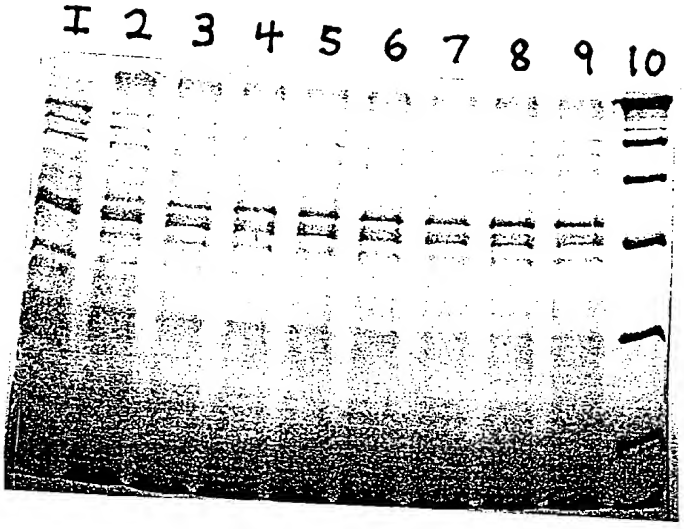
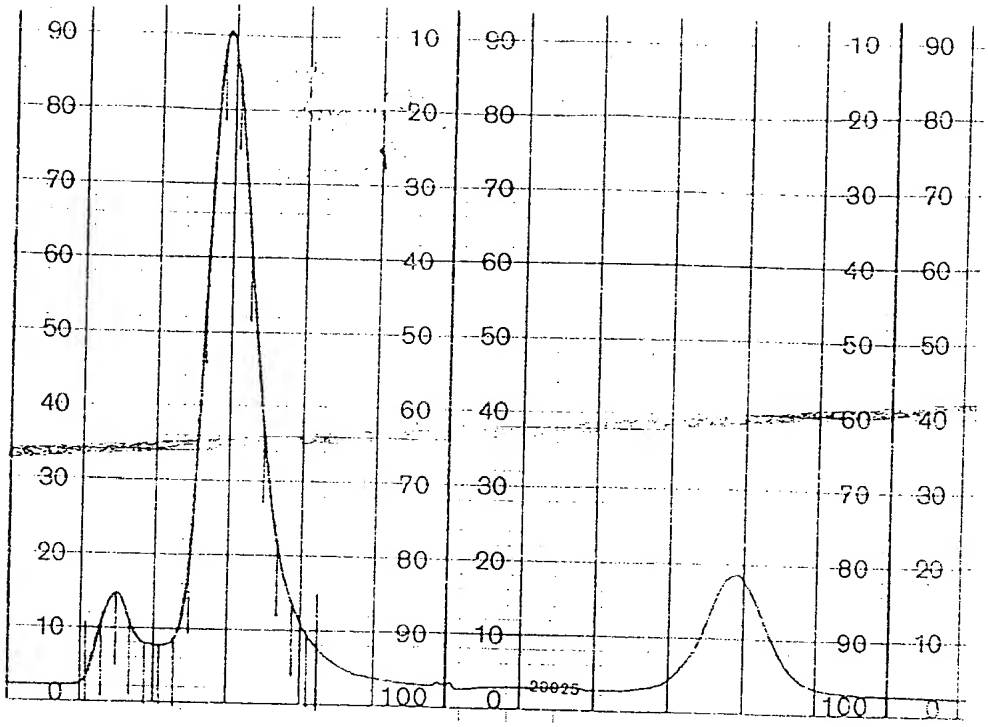



FIGURE 40